Survival of Salmonellae During Pepperoni Manufacture

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Survival of salmonellae in artificially contaminated beef-pork mixtures (approximately 10^4 salmonellae/g) was studied in pepperoni prepared by either a natural flora or lactic starter culture fermentation or in nonfermented sausages. The pepperoni did not become salmonellae free during the usual commercial 15- to 30-day drying period. *Salmonella dublin* was present in all products, fermented or unfermented, after 42 to 43 days of drying. At a lower level of contamination, 10^3/g, *S. dublin* could not be recovered from starter culture-fermented pepperoni after 14 days of drying but persisted in the natural flora-fermented sausage. *S. typhimurium* (initial count, 10^4/g) was absent after 42 days of drying when starter culture was used to ferment the pepperoni, but was still present in the natural flora-fermented and unfermented products. *S. dublin*, host adapted to cattle, or *S. choleraesuis*, host adapted to swine, had similar survival patterns in beef, pork, or beef-pork pepperoni. Heating salmonellae-contaminated beef-pork pepperoni (after fermentation but before drying) to an internal temperature of 60 C (trichinae inactivating) eliminated the food-borne pathogen from the sausage product.

Dry and semidry fermented sausages have rarely been implicated in food poisoning. However, an outbreak of salmonellosis has been attributed to the presence of *Salmonella choleraesuis* in Italian dry salami (5), and, in the United States, an incident of staphylococcal food poisoning was traced to Genoa sausage (2). In the latter case, the sausage contained coagulase-positive staphylococci with enterotoxin A; salmonellae were found also.

Most workers have approached the study of the survival of salmonellae in fermented sausages by artificially contaminating the product at some step during manufacture and then following the number of survivors in samples taken during the remainder of the processing operations. The fate of salmonellae during the preparation and storage of "isterband," a Swedish fermented sausage (6), thuringer (3), dauerwurst (11), and Lebanon bologna (9) has been studied in this way. In 1972, pepperoni was implicated in a food poisoning outbreak; however, the causative agent was not identified (1). This food poisoning case prompted us to study the potential for survival of selected salmonellae during the processing of pepperoni.

MATERIALS AND METHODS

The procedures used for pepperoni manufacture and techniques used for the determination of pH, acid content, and water activity (A_w) have been described previously by Palumbo et al. (S. A. Palumbo, L. L. Zaika, J. C. Kissinger, and J. L. Smith, J. Food Sci., in press).

S. dublin, *S. typhimurium*, and *S. choleraesuis* were used as contaminants in different lots of pepperoni sausages. A 20-h culture of the test organisms grown at 37 C in tryptic soy broth (Difco) was diluted with distilled water to give the appropriate concentration of cells. The diluted culture was mixed into the meat by hand, and then the sausage mixture was stuffed into 55-mm fibrous casings. Fermentation of the pepperoni mixture was carried out either by encouraging the natural lactic microflora through the aging of meat containing 3% NaCl for 10 days at 5 C (7) or by the use of commercial Lactacel MC starter culture (Merck and Co., Rahway, N.J.), a mixture of *Lactobacillus plantarum* and *Pediococcus cerevisiae*. The starter culture was used according to the recommendation of the manufacturer except that a straight nitrate cure of 1.2 g of $NaNO_3$/kg of meat was employed instead of nitrite. Unfermented pepperoni prepared from nonaged meat and without the addition of starter culture were processed by the procedures used for the fermented sausages.

In those experiments that investigated the influence of a "trichinae cook" on the survival of salmonellae, the contaminated pepperoni were heated in a smokehouse set at 87.8 C (190 F), dry bulb, and 76.7 C (170 F), wet bulb. The sausages were removed when the internal temperature reached 60 C (140 F), quartered with a sterile knife, placed in plastic bags, and immersed immediately in an ice bath. The quartered sausages were kept refrigerated until assayed for salmonellae. Determination of the viable salmonellae count has been described in a previous publication (8).

RESULTS

The influence of *Salmonella* serotype as well as the type of meat used to prepare pepperoni were studied to determine the survival of salmonellae during processing. Beef, pork, or beef-pork blends were prepared, and aliquots were contaminated with *S. dublin* (host adapted for cattle), *S. typhimurium*, or *S. choleraesuis* (host adapted for swine), respectively. The sausages were fermented by the natural lactic flora. These *Salmonella* serotypes were selected to determine whether a host-adapted serotype would behave differently from a non-host-adapted serotype in the three types of meat. However, there was very little difference in the survival patterns in pepperoni of the three *Salmonella* serotypes regardless of the meat type used. Since all commercial pepperoni are prepared from beef-pork mixtures (Palumbo et al., J. Food Sci., in press) and since there was no difference in *Salmonella* survival in pepperoni prepared from different meats, a beef-pork (1:1) mixture was used in all subsequent experimentation.

The survival of *S. dublin* and *S. typhimurium* in starter culture-fermented, natural flora-fermented, and unfermented pepperoni made from a mixture containing equal amounts of beef and pork is shown in Tables 1, 2, and 3.

TABLE 1. *Survival of salmonellae in beef-pork[a] pepperoni prepared with starter culture*

Day	pH	% Acid[b]	A_w[c]	No. of microorganisms/g of sausage	
				S. dublin	*S. typhimurium*
Fermentation[d]					
0	6.1	0.24		4.5×10^4	8.2×10^3
1	4.5	0.72		8.8×10^3	4.5×10^2
Drying[e]					
1	4.5	0.71	0.960	3.3×10^4	1.8×10^3
8	4.5	1.01	0.935	2.2×10^4	1.2×10^2
15	4.6	1.04	0.885	6.1×10^2	6.0×10^1
22	4.6	1.18	0.875	1.6×10^2	9.3
29	4.6	1.12	0.828	2.2×10^2	0.03
43	4.6	1.14	0.798	2.4×10^1	0.0[f]

[a] The sausages contained equal amounts of beef and pork.
[b] Percentage of acid: titratable acidity expressed as grams of lactic acid/100 g of sausage.
[c] A_w, Water activity.
[d] Conditions for fermentation: 35 C and 85% RH.
[e] Conditions for drying: 12 C at 60 to 65% RH.
[f] Bacterial numbers less than 0.03/g were expressed as zero.

TABLE 2. *Survival of salmonellae in beef-pork[a] pepperoni prepared by natural flora fermentation*

Day	pH	% Acid[b]	A_w[c]	No. of microorganisms/g of sausage	
				S. dublin	*S. typhimurium*
Fermentation[d]					
0	6.2	0.23		3.5×10^4	1.4×10^4
1	5.5	0.36		4.2×10^4	1.3×10^3
2	5.1	0.47	0.978	8.0×10^3	2.5×10^3
Drying[e]					
7	4.9	0.74	0.941	2.0×10^4	3.0×10^2
14	5.1	0.83	0.885	1.5×10^4	1.2×10^2
21	5.0	0.84	0.878	7.5×10^3	4.6×10^1
28	5.0	0.88	0.853	6.0×10^2	2.1
42	5.0	0.97	0.805	1.0×10^2	0.04

[a] The sausages contained equal amounts of beef and pork.
[b] Percentage of acid: titratable acidity expressed as grams of lactic acid/100 g of sausage.
[c] A_w, Water activity.
[d] Conditions for fermentation: 35 C and 85% RH.
[e] Conditions for drying: 12 C at 60 to 65% RH.

TABLE 3. *Survival of salmonellae in unfermented beef-pork[a] pepperoni*

Days	pH	% Acid[b]	A_w[c]	No. of microorganisms/g of sausage	
				S. dublin	*S. typhimurium*
Incubation[d]					
0	6.1	0.24		4.1×10^4	8.9×10^3
1	6.1	0.28		3.4×10^6	2.4×10^5
2	6.0	0.30	0.993	7.3×10^5	2.2×10^4
Drying[e]					
7	5.8	0.52	0.973	2.1×10^5	1.2×10^5
14	5.8	0.50	0.896	6.0×10^5	1.8×10^4
21	5.8	0.53	0.878	4.1×10^5	4.5×10^3
28	5.7	0.62	0.855	1.2×10^5	2.8
42	5.7	0.70	0.828	1.3×10^6	0.07

[a] The sausages contained equal amounts of beef and pork.
[b] Percentage of acid: titratable acidity expressed as grams of lactic acid/100 g of sausage.
[c] A_w, Water activity.
[d] Conditions of incubation: 35 C and 85% RH.
[e] Conditions for drying: 12 C at 60 to 65% RH.

The pepperoni that had starter culture incorporated into the meat mixture showed a decrease in pH from 6.1 to 4.5 after 1 day of fermentation at 35 C and a decrease in viable numbers of both test organisms (Table 1). No viable *S. typhimurium* were detected after 29 days of drying, whereas *S. dublin* was still detected after 43 days of drying. The pH decrease in sausages fermented by the natural flora was less pronounced than in those fermented by starter culture (Table 2). The pH of the latter decreased from 6.1 to 5.1 in 2 days at 35 C. There was a decrease in the number of viable salmonellae during the fermentation period (Table 2), but after 42 days of drying viable *S. dublin* and *S. typhimurium* were still present. There was virtually no change in pH in the unfermented pepperoni after 2 days at 35 C; at 1 day, there was growth of both *Salmonella* serotypes followed by a decrease in growth on day 2 (Table 3). During drying the acid content increased, resulting in a slight lowering of the pH (from 6.0 to 5.7). There was no decrease in viable *S. dublin* within a drying period of 42 days; *S. typhimurium* decreased to a low level but was still detected at 42 days of drying (Table 3).

S. dublin was shown to be more resistant to the processing conditions involved in pepperoni manufacture than *S. typhimurium* (Tables 1, 2, and 3). In the initial experiments, a relatively high number (about 4×10^4/g) of *S. dublin* was added to the meat mixture used to make the pepperoni. When an initial inoculum of 1.7×10^3 *S. dublin*/g was used in natural flora-fermented pepperoni (pH 4.8 after a 2-day fermentation), there was still about 1 viable cell/g at the end of 42 days of drying. On the other hand, when the initial inoculum of *S. dublin* was 9.0×10^2/g in starter culture-fermented pepperoni (pH 4.6 at 1 day of fermentation), no *S. dublin* could be recovered after 14 days of drying.

Since pepperoni is usually made from pork-beef mixtures, the sausage or the pork must be given some treatment to inactivate trichinae which might be present. Trichinae can be inactivated by standardized freezing, heating, or drying. Large-scale industrial freezing of pork for use in pepperoni is impractical and it has been demonstrated that drying is not completely reliable in destroying salmonellae in pepperoni (Tables 1, 2, and 3). Therefore, the fate of salmonellae added to pepperoni subjected to temperatures that would inactivate trichinae was investigated. The data in Table 4 indicate that heating the sausage to an internal temperature of 60 C (140 F) destroyed *S. dublin* remaining after the fermentation step (both starter culture and natural flora). The data indicate that salmonellae in unfermented pepperoni were destroyed by heating also. Salmonellae were not detected after 14 days of drying, indicating that there was no recovery of cells that might have been only heat injured.

DISCUSSION

Pepperoni is a highly spiced, fermented, dried sausage characteristically prepared from pork or pork and beef. The fermentation is similar to that of Lebanon bologna (7), but pepperoni is less acidic and is subjected to a long drying period. In a previous study, we found that salmonellae (at a level of about 10^4/g) was unable to survive the Lebanon bologna processing conditions except occasionally when natu-

TABLE 4. *Effect of heating on the destruction of S. dublin in beef-pork pepperoni*

Condition	Fermentation by:				Unfermented	
	Starter culture[a]		Natural flora[b]			
	pH	No. of *S. dublin*/g	pH	No. of *S. dublin*/g	pH	No. of *S. dublin*/g
Before cooking	4.6	2.0×10^2	4.8	3.2×10^3	5.8	1.6×10^2
After cooking[c]	4.7	0.0[d]	4.9	0.0	6.1	0.0
Drying (14 days)[e]		0.0		0.0		0.0

[a] One day of fermentation at 35 C, 85% RH.
[b] Two days of fermentation at 35 C, 85% RH.
[c] The sausages were cooked to an internal temperature of 60 C (140 F).
[d] Bacterial numbers lower than 0.03/g were considered to be zero.
[e] After cooking, the sausages were placed in the dry room at 12 C and 60 to 65% RH.

ral flora fermentation was used (8). The data obtained in this study indicated that salmonellae were not consistently killed by the processing conditions that were used in the manufacture of pepperoni.

Fermentation with Lactacel MC starter culture was more effective than natural flora fermentation in destroying salmonellae. The same batch of meat was used to compare the effect of starter culture (Table 1) and natural lactic flora (Table 2) on the survival of *S. dublin* and *S. typhimurium* during processing. The pH of the pepperoni prepared with starter culture was lower than that prepared by the natural flora process, but viable *S. dublin* were still present after 42 days of drying with both types of fermentation. Viable *S. typhimurium* were present after 42 days of drying in pepperoni prepared with natural lactic flora but not with starter culture (Tables 1 and 2). When the initial inoculum of *S. dublin* was decreased approximately 50-fold (to about 10^3/g), no viable cells were found in pepperoni prepared with starter culture, but *S. dublin* was still present in sausages made by the natural flora fermentation (after 42 days of drying). Smith et al. (8) found that in Lebanon bologna contaminated with salmonellae starter culture fermentation was more effective than natural flora fermentation in eliminating the food pathogen from sausage.

S. dublin, host adapted to cattle, or *S. choleraesuis,* host adapted to swine, behaved similarly in pork, beef, or beef-pork pepperoni. Host adaptation did not predispose the *Salmonella* serotypes to differential survival depending on the meat type used to prepare the sausage.

The pH values of commercial pepperoni produced by nine different companies showed a wide range: 6.1 to 4.7. Three of the products ranged in pH from 5.6 to 6.1; three ranged from 5.1 to 5.3; and three ranged from 4.7 to 4.9 (Palumbo et al., J. Food Sci., in press). Since a sausage with a pH of 5.5 or above would not be considered a fermented product, a study of the survival of salmonellae in unfermented pepperoni was indicated. There was an increase in *S. dublin* during the processing period from an initial 4.1×10^4/g to 1.3×10^6/g after 42 days of drying; *S. typhimurium* decreased to a very low value but was till detectable at 42 days of drying (Table 3). Unfermented pepperoni is marketed and presumably preferred by some consumers. The data presented in Table 3 suggest that *Salmonella* serotypes similar to *S. dublin* would probably survive in high numbers in such unfermented products and thus lead to a contaminated sausage.

S. dublin was more resistant to destruction than *S. typhimurium* during the production of pepperoni (Tables 1, 2, and 3) and under the processing conditions for Lebanon bologna (8).

Since all commercial pepperoni contain pork (Palumbo et al., J. Food Sci., in press), a trichinae-inactivating heating step at the end of fermentation and before drying was investigated to determine if this would result in the destruction of salmonellae. An internal temperature of 60 C (140 F) in the sausage was effective in destroying *S. dublin* (Table 4).

Since salmonellae were not always killed during pepperoni processing (in the absence of heating), apparently the safety of commerical pepperoni is due to other factors such as low contamination levels, postproduction heating (as in pizza), or by differences between commercial pepperoni processing methods and ours. Surkiewicz et al. (10) found that 28% of 560 fresh pork sausage samples were contaminated with low numbers of salmonellae, whereas only 0.4% of 735 fresh beef patties contained salmonellae (9). Therefore, pepperoni made from beef and pork mixtures could be expected to be occasionally contaminated with salmonellae. Commer-

cial companies normally hold their pepperoni in the dry room for 15 to 30 days (4), and since the level of salmonellae, if present at all, is usually very low, the number of contaminants might be reduced to undetectable levels by the end of the drying period. Our data indicated a large reduction of salmonellae during fermentation and drying. However, in cases of high contamination levels, an unsafe product could result. Inclusion of a trichinae heating step of 60 C in commerical pepperoni production would ensure a *Salmonella*-free product.

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